



**HAL**  
open science

## **Kinetic study of some flavor and bioactive compounds during fermentation of *Parkia biglobosa***

Michel Esse, Tagro Guehi, Marc Lebrun, Gilles Morel, Joël Grabulos, Christian Mestres, Nawel Achir

### ► **To cite this version:**

Michel Esse, Tagro Guehi, Marc Lebrun, Gilles Morel, Joël Grabulos, et al.. Kinetic study of some flavor and bioactive compounds during fermentation of *Parkia biglobosa*. *Journal of Food Processing and Preservation*, 2022, 46 (10), 10.1111/jfpp.16888 . hal-03936396

**HAL Id: hal-03936396**

**<https://institut-agro-montpellier.hal.science/hal-03936396v1>**

Submitted on 9 Feb 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Kinetic study of some flavor and bioactive compounds during fermentation of *Parkia biglobosa***

Journal:	<i>Journal of Food Processing and Preservation</i>
Manuscript ID	JFPP-12-21-3307.R2
Wiley - Manuscript type:	Original Article (Direct Via EEO)
Date Submitted by the Author:	n/a
Complete List of Authors:	Esse, Michel Yavo; aLaboratoire de Biotechnologie et Microbiologie des Aliments, Université Nangui Abrogoua Guehi, Tagro S.; Univ Nangui Abrogoua Lebrun, Marc; International Centre in Agronomical Research for Development, Fruits and Citrus fruits Research Institute Morel, Gilles; CIRAD Montpellier-Occitanie Research Centre, Unité Mixte de Recherche QualiSud, F-34398 Montpellier, France Grabulos, Joël; CIRAD Mestres, Christian; CIRAD Achir, Nawel; Montpellier SupAgro
Keywords:	aroma compounds, short chain fatty acids, biogenic amines, kinetics

1  
2  
3 1 **Kinetic study of some flavor and bioactive compounds during fermentation of *Parkia***  
4  
5 2 ***biglobosa***

6  
7 3 **M. Esse <sup>a,b</sup>, T. Guehi <sup>a</sup>, M. Lebrun <sup>b,c</sup>, G. Morel <sup>b,c</sup>, J. Grabulos <sup>b,c</sup>, C. Mestre <sup>b,c</sup>, N. Achir <sup>b,\*</sup>**  
8  
9 4

10  
11 5  
12 6 <sup>a</sup>Laboratoire de Biotechnologie et Microbiologie des Aliments, UFR de Sciences et  
13  
14 7 Technologies des Aliments, Université Nangui Abrogoua

15  
16 8 <sup>b</sup>Qualisud, Univ Montpellier, Institut Agro, CIRAD, Avignon Université, Univ de La Réunion,  
17  
18 9 Montpellier, France.

19  
20 10 <sup>c</sup>CIRAD, UMR QualiSud, F-34398 Montpellier, France  
21  
22 11

23 12 \*Corresponding author: Nawel Achir. 1101, avenue Agropolis, 34090 Montpellier Cedex 5,  
24  
25 13 France.

26  
27 14 E-mail: [nawel.achir@supagro.fr](mailto:nawel.achir@supagro.fr)  
28  
29 15  
30  
31 16  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 17 **Abstract**  
4

5 18 Seeds of *Parkia biglobosa* also called African locust bean (ALBS) are used to produce a  
6  
7 19 condiment by subjecting them to cooking, alkaline fermentation and drying. The objective of  
8  
9 20 this work was to monitor the evolution of the composition of ALBS during spontaneous  
10  
11 21 fermentation. Results showed that the seeds at initial time contained mainly proteins (45%)  
12  
13 22 and lipids (36%). During fermentation, conversion of this initial composition took place to  
14  
15 23 produce ammonium, free amino acids and short chain fatty acids (SCFA) that accounted for 2,  
16  
17 24 10 and 1.5% db respectively which explains the typical flavor of fermented ALBS. This release  
18  
19 25 was also concomitant with biogenic amine production. Along with ammoniac and SCFA  
20  
21 26 release, pyrazines were the major volatile organic compounds. Composition modulation was  
22  
23 27 well represented by a Verhulst equation of which the dynamic could be modified by the initial  
24  
25 28 composition of the seed, the pre-cooking step, and the fermentation conditions (temperature,  
26  
27 29 aeration).  
28  
29 30

30 31 **Key words: soumbala, aroma compounds, short chain fatty acids, biogenic amines, kinetics,**

31 32 **Verhulst equation**  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 34 **Practical applications**  
4  
5 35  
6  
7  
8 36 For nutritional and sustainability reasons, re-integration of vegetable in general and legumes  
9  
10 37 in particular in our diet is a current trend. However, only dish that are appealing to consumers  
11 38 are concretely adopted. Fermentation of legumes is interesting in more than one way. It does  
12  
13 39 not involve heat, so thermo-labile molecules can be preserved and the financial and energetic  
14  
15 40 costs are low. Biological interesting molecules are produced from the metabolism of  
16  
17 41 microorganisms and among them, volatile organic compounds that contribute to enhance the  
18  
19 42 flavour of legume dishes. Soumbala is a meal and/or condiment obtained from the  
20  
21 43 fermentation of African locust bean (*Parkia biglobosa*) seeds (ALB), a legume widely spread in  
22  
23 44 west Africa. The final typical sensory properties are known to be due to the alkaline  
24  
25 45 fermentation step by *Bacillus* species. This paper presents the kinetics of release of various  
26  
27 46 compounds during spontaneous fermentation that may impact the sensory and nutritional  
28  
29 47 properties of the final product. More precisely, the dynamic of the production of free amino  
30  
31 48 acids, free fatty acids, volatile compounds and biogenic amines are presented and discussed  
32  
33 49 to give insights into the succession of important reactions to control the sensory and  
34  
35 50 nutritional quality of fermented ALBS. This paper shows that a lot can be done to better  
36  
37 51 understand and control the quality of FALBS but also similar protein-rich legumes to modulate  
38  
39 52 the quality of the final products.  
40  
41 53  
42 54  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 55  
4  
5 56  
6  
7 57  
8  
9 58  
10 59  
11  
12 60  
13  
14 61  
15  
16 62  
17  
18 63  
19  
20 64  
21  
22 65  
23  
24 66  
25  
26 67  
27  
28 68  
29  
30 69  
31  
32 70  
33  
34 71  
35  
36 72  
37  
38 73  
39  
40 74  
41  
42 75  
43  
44 76  
45  
46 77  
47  
48 78  
49  
50 79  
51  
52 80  
53  
54 81  
55  
56 82  
57  
58 83  
59  
60 84  
85  
86

## Introduction

Legumes are a sustainable source of protein and other bio-active compounds that are sensitive to temperature. Fermentation is a non-thermal preservation process that can be applied to legumes. In addition, previous studies showed that due to microbiological activity, the fermentation step increased certain B-group vitamins and decreased anti-nutritional factors widely found in legumes such as alpha-galactosides or phytates (Adeyemo & Onilude, 2013; Granito et al., 2002). Some fermented products are traditionally made from legumes such as soybean, locust bean seed, chickpea, black gram or peanut (Reddy, 2018). A variety of microorganisms are involved: lactic acid bacteria, yeasts, fungus or *Bacillus* producing a variety of tastes, aromas, textures and flavors. The fermented legumes are widely used for flavoring in Asia or West Africa (Odunfa, 1988).

Among them, a typical and popular West African condiment, called *soumbala*, *netetu*, *afitin*, *iru* or *dawadawa* depending on the country, is made by *Bacillus* fermentation of a protein-rich legume, the African locust bean seed (*Parkia biglobosa*). The traditional process of soumbala production consists of three main steps: cooking the seeds in boiling water for 12 to 24h to soften the hard outer shell and facilitate dehulling, cooking the cotyledons, spontaneous fermentation, and final stabilization by drying (Dakwa et al., 2005; Odunfa, 1985; Yusuf & Rahji, 2012). This production is still done on a small-scale and therefore hardly meets consumer demand in terms of quantity as well as sanitary and organoleptic quality (Odunfa, 1985; Omafuvbe et al., 2004). Organoleptic attributes of fermented African locust bean seed (FALBS) are the result of the production of hundreds of chemical compounds during fermentation (Akanni et al., 2018; Ouoba et al., 2005). These numerous reactions are due to the metabolism of *Bacillus* species that hydrolyzes proteins to free amino acids, ammonia and other basic nitrogenous compounds. This results in an increase of pH and a so-called "alkaline fermentation", which contributes to the stability of the final product and also to its typical ammoniacal flavor (Akanni et al., 2018; Amoa-Awua et al., 2014; Ouoba et al., 2003). *Bacillus* not only produce proteases but a diversity of other extracellular hydrolases that also react with the seed carbohydrates and lipids which may contribute to the flavors and aromas development (Odunfa, 1985).

1  
2  
3 87 Volatile organic compounds (VOCs) have already been studied in FALBS (Akanni et al., 2018;  
4  
5 88 Ouoba et al., 2005). A total of 116 to 125 VOCs were identified, belonging, by order of  
6  
7 89 quantities, to acids, pyrazines, nitrogen-containing compounds, aldehydes, alcohols, ketones,  
8  
9 90 esters, alkanes, alkenes, benzenes, phenols, and sulfur-containing compounds. Depending on  
10  
11 91 the relative concentration of the different VOCs, some consumers may or may not appreciate  
12  
13 92 the product (Amoa-Awua et al., 2014; Dakwa et al., 2005). Indeed, as for strong aromatic  
14  
15 93 products such as cheese or wine, the balance of the different molecules released and their  
16  
17 94 control is of major importance on the sensory quality and acceptance of the end-product. To  
18  
19 95 achieve this objective, knowledge of the impact of fermentation parameters on the  
20  
21 96 production dynamic of the different volatiles is a first step. Akanni *et al.* (2018) and Ouoba *et*  
22  
23 97 *al.* (2005) worked on the influence of *Bacillus* strains on the typology of final aroma  
24  
25 98 compounds. Akanni *et al.* (2018) found that *B. subtilis* and *B. licheniformis* contributed to  
26  
27 99 volatile acids, *B. cereus* to aldehydes and alcohols, and *B. amyloliquafaciens* produced sulfur-  
28  
29 100 containing compounds. Another way to control VOCs release is to modify the processing  
30  
31 101 conditions. Amoa-Awua *et al.* (2014) added humectant molecules like NaCl or glycerol during  
32  
33 102 fermentation to limit *Bacillus* populations. They also tested the effect of steaming after  
34  
35 103 fermentation. Both treatments applied significantly reduced the pungent odor because of a  
36  
37 104 decrease in ammonia content. They found that the VOCs associated with the most undesirable  
38  
39 105 sensory descriptors were ammonia, phenols, indoles, and volatile SCFA (Amoa-Awua et al.,  
40  
41 106 2014; Delgado et al., 2011). On other fermented or ripened products such as cheese, authors  
42  
43 107 found that SCFA had a major sensory impact because of their low odor threshold (Delgado et  
44  
45 108 al., 2011; Faccia et al., 2018; Noronha et al., 2008).

46  
47 109 In addition to VOCs some non-volatile compounds may significantly contribute to the  
48  
49 110 organoleptic properties of FALBS. Indeed, previous studies showed that FALBS contained  
50  
51 111 significant amounts of free amino acids (AA) and FFA that can impact its sensory properties  
52  
53 112 (Esse et al., 2021; Odunfa, 1985; Omafuvbe et al., 2004). Indeed, Zhang et al. (2020) showed  
54  
55 113 that AA such as serine, aspartic and glutamic acids, glycine, alanine, proline, cysteine,  
56  
57 114 methionine, and lysine, significantly and positively influenced the sensory descriptors of  
58  
59 115 chicken broth and muscle (Zhan et al., 2020). Other bioactive molecules that may come from  
60  
116 AA are biogenic amines (BA). Although BA are present in living cells and contribute to different  
117  
118 physiological functions, high consumption via food can cause intoxication. Secondly,  
elevated levels of biogenic amines can contribute to the pungent or objectionable odor of

1  
2  
3 119 fermented products (Cohen et al., 2014; Dekeirsschieter et al., 2009). The link between  
4  
5 120 biogenic amines and the sensory profile of fermented products was studied in wine. It was  
6  
7 121 proved that agmatine and  $\beta$ -phenylethylamine were correlated to a positive sensory profile,  
8  
9 122 while putrescine, tyramine, histamine, cadaverine and spermidine were linked to negative  
10  
11 123 descriptors (Ordóñez et al., 2017). Finally, FFA like myristic, palmitic, stearic, and eicosanoic  
12  
13 124 acids can also affect the flavor of lipid-rich foods (Zhan et al., 2020).  
14  
15 125 Alkaline fermentation of legumes in general, and that of African legumes in particular, is poorly  
16  
17 126 studied. However, the use of fermented legumes contributes to food sustainability and flavor  
18  
19 127 diversity. Therefore, it is important to gain insight into their production and into the reactions  
20  
21 128 that takes place during fermentation that impact their sensory and nutritional quality. Because  
22  
23 129 fermented African locust bean seeds is a condiment, the previous studies mainly focused on  
24  
25 130 VOCs profile at the end of processing. However, a better understanding of volatile and non-  
26  
27 131 volatile compounds release throughout the fermentation process would be very helpful for  
28  
29 132 producers to better control the quality of fermented legumes. Therefore, the objective of this  
30  
31 133 paper is to describe the kinetics of bioactive compound production during the spontaneous  
32  
33 134 fermentation of ALBS.

## 34 136 **Material and methods**

### 38 138 **Plant material**

39  
40 139 The harvest of African locus bean seeds takes place from March to May. Seeds are dried and  
41  
42 140 are available on local markets till the next harvest season. The seeds used for this study were  
43  
44 141 bought in the region of Khorogo, where most Soumbala production is located, in the north of  
45  
46 142 Côte d'Ivoire.

### 49 144 **Fermentation of African locust bean seeds**

50  
51 145 Before fermentation, ALBS were first put into boiling water at a ratio seed/water of 1/3 for  
52  
53 146 24h over a traditional wood fire to prepare dehulling. The seeds were dehulled in a laboratory  
54  
55 147 mortar. Dehulled raw seeds were cooked in boiling water at a ratio seed/water of 1/1 for 1  
56  
57 148 hour. Spontaneous fermentation of 500 g of cooked cotyledons was done in jute bags placed  
58  
59 149 in small baskets in a chamber regulated at 40 °C for 48 h. Samples were collected at 0, 12, 24,



1  
2  
3 150 **36 and 48 h of fermentation**. To stabilize them, fermented seeds were dried in an oven at 50  
4  
5 151 °C for 12 h. The whole process was repeated. A total of 17 samples were collected throughout  
6  
7 152 the production process and more precisely: 3 samples of raw seeds, 4 samples of cooked seeds  
8  
9 153 at time 0 of fermentation, 2 samples after 12, 24 and 36 h of fermentation and finally 4 at the  
10  
11 154 end of 48 h of fermentation. All samples were dried and stored at -18 ° C.

12 155

### 14 156 **Proximate composition**

16 157 **The dry matter content was determined by oven drying at 105°C to a constant weight. The**  
17  
18 158 **nitrogen content of all samples was determined using the Kjeldhal method and a conversion**  
19  
20 159 **factor of 5.85 was used according to AOAC 979.09. Lipid content was done by Soxhlet**  
21  
22 160 **extraction according to AOAC 963.15. Total free fatty acids of the lipids obtained by Soxhlet**  
23  
24 161 **extraction was determined by titrimetry according AOAC Cd 3d-63. Ashes were obtained**  
25  
26 162 **gravimetrically after incineration of the sample at 550°C for approximately 7h according AOAC**  
27  
28 163 **923.03. Total carbohydrates were estimated by subtracting the measured protein, fat, ash,**  
29  
30 164 **and water from the total weight.**

31  
32 165

### 34 166 **Free and Total Amino Acid**

36  
37 167 **Free amino acids were analyzed following the method used by (Moore et al., 1958), with**  
38  
39 168 **modifications. Briefly, 15 mg of samples were weighed and placed in a sealable test tube. To**  
40  
41 169 **this, 50 µl of internal standard Norleucine (25 µM) and 4.95 mL of citrate buffer (pH 2.2) were**  
42  
43 170 **added. The solution was mixed for 1 h on a rotational shaker. For total amino acid**  
44  
45 171 **determination, 10 to 20 mg of sample were weighed in a Schlenk tube and 50 µl of 25 µM**  
46  
47 172 **norleucine and 450 µL of 4 M methanesulfonic acid were added. The tube was flushed with**  
48  
49 173 **nitrogen, closed and heated at 150°C for 2 h. After cooling, 450 µL of 4 M NaOH was added to**  
50  
51 174 **the hydrolysate, which was diluted to 5 mL with a loading buffer (citrate buffer at pH 2.2).**  
52  
53 175 **Sample extracts for free and total amino acid analysis were filtered using a 0.45 µm membrane**  
54  
55 176 **filter and injected into the amino acid analyzer (Biochrom 30+, Biochrom, France), using a**  
56  
57 177 **lithium cation exchange resin column and ninhydrin as a detection compound. All total and**  
58  
59 178 **free amino acid contents were determined except total tryptophan, which is the most fragile**  
60

1  
2  
3 179 amino acid and is destroyed by the extraction procedure. Amino acid standards were also run  
4  
5 180 in a similar way as the samples.  
6  
7  
8 181

### 10 182 **Biogenic amines**

12 183 Analysis of biogenic amines was carried out based on the procedure described in (Yoon et al.,  
13 184 2015). Briefly, 20 mL of 0.4 M perchloric acid were added to 5 g of the samples, and the  
14 185 mixture was homogenized using a vortex mixer (Vortex-Genie, USA), reacted in a cold  
15 186 chamber at 4 °C for 2 h, and centrifuged at 3000 ×g at 4 °C for 10 min. The supernatant was  
16 187 collected, and the residue was extracted again with an equal volume of 0.4 M perchloric acid.  
17 188 Both supernatants were combined, and the final volume was adjusted to 50 mL with 0.4 M  
18 189 perchloric acid. The extract was filtered then, derivatization of biogenic amines was carried  
19 190 out as follows: one mL of extract (or standard solution) was mixed with 200 µL of 2 M sodium  
20 191 hydroxide and 300 µL of saturated sodium bicarbonate solution. Two mL of dansyl chloride  
21 192 solution (10 mg/mL) prepared in acetone were added to the mixture, which was then  
22 193 incubated at 40 °C for 45 min. Residual dansyl chloride was removed by adding 100 µL of 25%  
23 194 ammonium hydroxide. After incubation for 30 min at 25 °C, the volume of mixture was  
24 195 adjusted with acetonitrile to 5 mL. Finally, the mixture was centrifuged at 3000 × g for 5 min,  
25 196 and the supernatant was filtered through 0.2 µm-pore-size filters (Millipore Co., USA). The  
26 197 filtered supernatant was kept at -25 °C before HPLC injection. An HPLC unit equipped with a  
27 198 UV-Vis detector (Dionex Ultimate 3000, France) was employed. An ACE C18 column (5µm  
28 199 particle size, 250 mm × 4.6 mm, ACE, France) was used for the separation of the biogenic  
29 200 amines, and the samples were eluted with a step gradient of ammonium acetate (0.1 M  
30 201 solvent A) and acetonitrile (solvent B) as the mobile phases at the flow rate of 1 mL/min. The  
31 202 program was set for a linear gradient starting from 50% of solvent B to reach 90% of the  
32 203 solvent at 19 min. The sample volume injected was 10 µL and monitored at 254 nm. Standard  
33 204 stock solutions of biogenic amines, putrescin, cadaverine, histamine and β-phenylethylamine  
34 205 were separately prepared at 10 mg/L concentration in distilled water.  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

### 207 **Volatile compound**

208 Firstly, 1.5 g of ground sample was placed and sealed in 10 mL headspace vials with 1.4 mg of  
209 the internal standard trans-2-octenal and extracted by headspace solid phase micro extraction  
210 (HS-SPME) (Corrales et al., 2017). The extraction was performed at 60°C with 15 min  
211 incubation and 30 min trapping and shaking using a polydimethylsiloxane/divinylbenzene  
212 fiber (PDMS/DVB 65 mm, SUPELCO, Bellefonte, PA, USA). The volatile compounds were  
213 identified using gas chromatography/mass spectrometry. A tandem gas chromatograph  
214 6890/MSD 5973 N (Agilent Technologies, Palo Alto, USA) and a Gerstel autosampler MPS-2  
215 were used. A non-polar capillary column DB-5MS (5% diphenyl, 95% dimethyl siloxane, 30 m  
216 × 0.250mm × 0.25 µm) and a polar capillary column DBWax UI (polyethylene glycol 30 m ×  
217 0.25mm × 0.25 µm), both from J&W Scientific (Folsom, CA, USA), were used with a carrier gas  
218 hydrogen flow rate of 1.2 mL/min. Then, fiber was desorbed at 250 °C in splitless mode and  
219 was eluted with the following temperature program: 3 °C per min from 40 °C to 170 °C, then  
220 10 °C per min up to 240 °C and held for 10 min. Mass spectrum were recorded in EI+ mode at  
221 70 eV within a range of 40 to 350 Da. Analyzer and source temperatures were 150°C and  
222 250°C, respectively. Data were analyzed with Masshunter version B. 06.00 (Agilent  
223 Technologies, Palo Alto, USA). The identification of peaks was performed by comparing their  
224 mass spectra with those from the NIST 2011 (National Institute of Standard Technology)  
225 database. Co-injection on both columns of alkanes from C8 to C20 (Sigma-Aldrich, St. Louis,  
226 MO, USA) was used to calculate Kovats retention index (RI) for comparison with those found  
227 on the Flavornet, Pherobase and NIST websites.

228

### 229 **Statistical analysis**

230 One-way ANOVA was used to analyze the effect of fermentation time on the macronutrient  
231 composition in FALBS. If significantly different, means were further compared using Tukey's  
232 test. One-way ANOVA was also done to select discriminant volatile compounds before  
233 principal component analysis (PCA). Statistical analyses were performed with Statistica®  
234 (StatSoft Inc., USA).

235

### 236 **Kinetic modelling**

237

238 Microbiological growth during fermentation is assumed to be logistic and that production of  
239 different bioactive compounds produced by microorganisms is often linearly correlated to  
240 population, therefore we chose the usual Verhulst logistic function to represent production  
241 (Peleg et al., 2007; Yang et al., 2011):

$$242 \quad [X](t) = [X]_{\infty} \times \frac{1}{1 + L \times \exp^{(-k \times t)}} \quad \text{eq. (1)}$$

243 Where  $[X]$  is the bioactive compound in g or mg.kg<sup>-1</sup> (DB) at the time  $t$  (h) of fermentation,  $[X]_{\infty}$   
244 is the asymptotic value of the curve that can be assimilated to the maximal concentration of  
245  $X$  at the end of fermentation,  $k$  is the logistic growth rate or steepness of the curve while  $L$   
246 refers to its sigmoid shape.

247 For each compound, the constants  $[X]_{\infty}$ ,  $L$  and  $k$  were identified by non-linear regression  
248 thanks to the Levenberg-Marquardt minimization procedure using the Matlab® software (The  
249 Mathworks Inc., Natick, Mass, USA).

250

### 251 **Parameter standard deviation**

252 The standard deviations on identified parameters were determined via the method of  
253 bootstrapping simulations (Huet et al., 1992). The principle is the generation of a high number  
254 (500) of resamples of the observed dataset (and of equal size to the observed dataset) added  
255 with a Gaussian perturbation within the experimental standard deviations. The mean and  
256 standard deviations were estimated for each constant rate from the 500 values.

257

## 258 **Results**

259

### 260 **Macro-composition evolution during fermentation**

261 Raw ALBS were composed of 5% ashes, 25 % total carbohydrates, 25 % lipids and 45 %  
262 proteins, on a dry basis (DB). The cooking step resulted in significant leaking of carbohydrates  
263 that decreased from 25 to 15% DB while proportions of lipids increased to 36% and proteins  
264 remained stable. The cooking step also resulted in hydration of the dry seeds up to 60 to 70%,  
265 which was necessary for fermentation (Esse et al., 2021).

266 Fig. 1 represents the evolution of these macro-constituents on a DB during fermentation. In  
267 general, the composition of dry matter was quite stable. The major constituent being proteins,

1  
2  
3 268 then lipids and finally the rest of dry matter is **composed mainly of** carbohydrates and  
4  
5 269 minerals. A slight change came from an increase of lipids from 35 to 42%. This increase was  
6  
7 270 due to the preferential consumption of carbohydrates and proteins by microorganisms during  
8  
9 271 fermentation and was already observed in other studies about ALBS fermentation (Azokpota  
10  
11 272 et al., 2006; Esenwah & Ikenebomeh, 2008). However, within each nutrient category,  
12  
13 273 especially proteins, transformations occurred because of microorganism actions.  
14  
15 274

#### 16 275 **Main changes of biological components during fermentation**

17  
18 276 Figure 2 a) b) and c) show the release of free amino acids (AA), ammonium and free fatty acids  
19  
20 277 (FFA) respectively. For the three indicators, the logistic model of Verhulst fitted well the  
21  
22 278 experimental data. Therefore, we can assume that their production was linked with the usual  
23  
24 279 shape of microorganism growth of *Bacillus*. Production of AA,  $\text{NH}_4^+$  and FFA started after about  
25  
26 280 10 h of fermentation. After an exponential increase, the maximal concentration was achieved  
27  
28 281 after about 25 h for FFA, and 35 h for  $\text{NH}_4^+$  and FFA. AA and  $\text{NH}_4^+$  are released by protein  
29  
30 282 degradation, while FFA comes from triglyceride hydrolysis. The maximal value of AA was  
31  
32 283 almost 12 % which represented one third of the proteins. In their experiments, (Odunfa, 1985)  
33  
34 284 found a level of 8% of AA at the end of fermentation. FFA reached 7% which represented 20%  
35  
36 285 of the total lipids. The equation parameters of Verhulst are presented in Figure 3. The k values  
37  
38 286 (equation 1) were higher for AA, then ammonium and lastly FFA which is consistent with our  
39  
40 287 observations of proteins being consumed before lipids. The sigmoid curvature of free amino  
41  
42 288 acids and ammonium release (L) was also higher than that of FFA. Therefore, we can say that  
43  
44 289 during ALBS fermentation, the release of free amino acids occurred first followed by  
45  
46 290 ammonium because of protein and amino acid catabolism and in a second time lipid  
47  
48 291 catabolism occurred with the release of FFA. Odunfa (1985) showed that protease activity was  
49  
50 292 higher than that of lipase. He also represented amino acid release of which the trend appeared  
51  
52 293 logistic as well. Release of AA,  $\text{NH}_4^+$  as well as FFA influence the flavor of fermented ALBS.  
53  
54 294 Indeed, some AA can contribute to the umami taste (Lioe et al., 2010), while  $\text{NH}_4^+$  induces the  
55  
56 295 strong ammonia smelling characteristic of FALBS (Amoa-Awua et al., 2014). At last, FFA are  
57  
58 296 major contributors to the flavor produced from lipolysis (Noronha et al., 2008). FFA and  
59  
60 297 particularly palmitic, stearic and eicosanoic acids that are present in FALBS can produce a fatty  
298  
299 298 flavor (Zhan et al., 2020). Previous studies showed that these three fatty acids were the most  
important after linoleic and oleic acids in ALBS (Esse et al., 2021). In addition to this direct

1  
2  
3 300 contribution to the sensory properties of FALBS, AA and FFA are precursors of other bioactive  
4  
5 301 compounds shown in Figure 2 d) and e) which are biogenic amines (BA) and short chain fatty  
6  
7 302 acids (SCFA). Those two compounds also behaved according to the Verhulst model and were  
8  
9 303 produced after amino acid release. Indeed, BA and SCFA growth parameters were close with  
10  
11 304  $k$  equal to 0.16 and 0.15 respectively. Therefore, they could be considered as secondary  
12  
13 305 products of amino acids. Mohamed et al. (2001) also showed that BA were produced after AA,  
14  
15 306 which may be interesting as a tool to control fermentation or ripening time of Egyptian salted-  
16  
17 307 fermented fish (Mohamed et al., 2001; Rabie et al., 2009). SCFA are fatty acids that have one  
18  
19 308 to seven carbons. SCFA maximal content at the end of fermentation was 1.7%. SCFA could  
20  
21 309 come from sugars, proteins and lipids (Akanni et al., 2018; Dursun et al., 2017). These  
22  
23 310 molecules confirm the proteolysis and lipolysis that occur during ALBS fermentation and are  
24  
25 311 known to contribute significantly to the acidic, sour, and pungent flavor of FALBS (Odunfa,  
26  
27 312 1985; Omafuvbe et al., 2004). In cheese, a rich protein and lipid product, SCFA contribute  
28  
29 313 greatly to the flavor and its typicality and could be produced by anaerobic bacterial  
30  
31 314 fermentation linked to the metabolism of *Bacillus* (Pasvolosky et al., 2014).

### 315 316 **Release Kinetics of amino acids**

317 The composition in amino acids of ALBS was presented in a previous article (Esse et al., 2021).  
318 The major amino acids were glutamic acid (Glu) at 20 %, followed by aspartic acid (Asp) at  
319 10%, leucine (Leu) and lysine (Lys) at both 7%, arginine (Arg) and valine (Val) at 6%, alanine  
320 (Ala), proline (Pro), glycine (Gly), phenylalanine (Phe) at 5%, serine (Ser), isoleucine (Ile),  
321 tyrosine (Tyr), histidine (His), threonine (Thr) at 2 to 4%, and cysteine (Cys), methionine (Met)  
322 and ornithine (Orn) at concentrations <2%. Figure 4 shows the release of the different free  
323 amino acids. Among all amino acids of the legume, only 13 were significantly released and/or  
324 observable during fermentation. To be easily represented, the amino acids were presented on  
325 three different figures 4 a), b) and c) in g/100g DB. For all amino acids, the Verhulst function  
326 fitted the experimental data well which is consistent with previous observations (Odunfa,  
327 1985). Among acid AA, Glu was the most produced reaching nearly 1.5 % DB which is  
328 consistent with the initial composition of the seed. This observation was already done and  
329 Odunfa (1985) stated that the release of Glu was particularly interesting in FALBS since Glu is  
330 a flavor enhancer. The author explained this high release by the ability of *Bacillus* species to  
331 accumulate Glu extracellularly. Cyst and Met were among the least produced which is

1  
2  
3 332 coherent with the low values of sulfur AA in legumes (Krishnan, 2005). However, Asp release  
4  
5 333 was as low as the sulfur AA which was not consistent with the initial composition of the seed.  
6  
7 334 Çalik et al. (2000) showed that *Bacillus licheniformis* was able to express the protease activity  
8  
9 335 differently as a function of the oxygenation condition. In addition, the types of amino acids  
10  
11 336 that were excreted to the fermentation medium also depended on the oxygenation  
12  
13 337 conditions. The least release of amino acids of the aspartic acid-group could be explained by  
14  
15 338 the need of this AA in *Bacillus* cells and a low asparagine synthetase expression (EC. 6.3.1.4)  
16  
17 339 or high-aspartate kinase (EC. 2.7.2.4) activity (Çalik et al., 2000). Another non-observable AA  
18  
19 340 was Arg, although it was the fifth in terms of initial quantity. However, Çalik et al. (2000) did  
20  
21 341 not observe its non-excretion. With Cys (Fig. 4a), Phe and Lys (Fig 4c), Leu, Ile, Val, and Trp (fig  
22  
23 342 4 b) are essential amino acids. Leu, Val, Phe, and Lys releases were close to 1% at the end of  
24  
25 343 fermentation. As their initial content in the seed was about 3% DB, nearly 30% of the amino  
26  
27 344 acids were in their free form at the end of fermentation which may be beneficial for a good  
28  
29 345 assimilation of these essential amino acids (Ito et al., 2018; LU et al., 2017). Odunfa (1985)  
30  
31 346 also observed a high release of valine. Recommended daily amounts for these essential AA  
32  
33 347 are 25 to 40 mg/kg/day as a function of AA (FAO/WHO/UNU, 2007). Therefore, the need for  
34  
35 348 people weighing 60-80 kg is 1.5 to 3 g/day. Assuming one portion of this condiment weighs 20  
36  
37 349 g, a meal can provide 20 to 30% of recommended daily intake. The non-essential AA Tyr also  
38  
39 350 reached 1% while His and Orn reached 0.5%. At the end of fermentation, these AA were at 80,  
40  
41 351 40 and 60 % in their free form respectively. Therefore, these compounds may be not crucial  
42  
43 352 for *Bacillus* cell metabolism or were favorably excreted.

353

#### 354 **Release Kinetics of biogenic amines**

355 The main BA found and presented in Fig. 4d were by order of importance spermidine (Sper),  
356  $\beta$ -phenylethylamine (Phen), histamine (Hist), putrescine (Put), and cadaverine (Cad). Sper  
357 comes from from Arg, Phen from Phe, Hist from His, Put from Arg and Cad from Lys. The final  
358 levels were 200, 90, 65, 30, 40 ppm respectively leading to a total close to 400 ppm (Fig. 4).  
359 This total value reached the limit defined by the European regulation for products having  
360 undergone an enzymatic maturation treatment. Therefore, BA production must be monitored  
361 carefully during ALBS fermentation. The high content of Sper may be due to the high  
362 conversion from Arg, and may explain the absence of this AA in Fig 4. An intermediary  
363 compound of this conversion can be Put. In cured ham, Arg content was low due to its

1  
2  
3 364 hydrolysis to ammonia and Orn, followed by decarboxylation to Put that was most largely  
4  
5 365 present in aged hams (Virgili et al., 2007). Also, it was suggested that the amounts of Put and  
6  
7 366 other BA in hams could be inversely related to the levels of their amino acid precursors and  
8  
9 367 that some BA generation may be regarded as the last step of the decarboxylation-deamination  
10  
11 368 mechanism starting with Arg (Alfaia et al., 2004). For the kinetic aspect, the most rapidly  
12  
13 369 produced free AA were Phe, His, Met, Glu, and Ile with  $k > 0.5$  (Figure 3). Regarding BA, the  
14  
15 370 most rapidly produced were Hist, Cad, and Put respectively and their maximal concentration  
16  
17 371 was reached at the early stage of fermentation, that is to say after about 10 hours. Hist  
18  
19 372 production was highly correlated to Hist release. Cad was produced more rapidly than its  
20  
21 373 precursor but rapidly reached a maximum. Put was probably produced very rapidly from Arg,  
22  
23 374 but the quantity of this BA was low, probably due to its rapid conversion to Sper. Phen and  
24  
25 375 Sper were produced in the second part of fermentation and their maximal value was reached  
26  
27 376 after 30 hours of fermentation. It is important to remark that Sper and Hist did not fit Verhulst  
28  
29 377 model after reaching their maximal content. Indeed, their content decreased suggesting a  
30  
31 378 potential degradation. This reaction may be conducted by microorganisms including certain  
32  
33 379 strains of *Bacillus* (Alvarez & Moreno-Arribas, 2014; Lee et al., 2016).

380

### 381 **Release Kinetics of Short chain fatty acids**

382 The most odorant SCFA are present in Fig 5a. They were composed of formic (C1), acetic (C2),  
383 butyric (C4) and the branched 3-methylbutanoic acid also called isovaleric acid (C5). Their  
384 respective odor threshold in air in ppm is  $8, 5 \cdot 10^{-3}, 3 \cdot 10^{-3}$  and  $8 \cdot 10^{-5}$  respectively. Formic acid  
385 odor is described as sharp acetic and fruity fermented, that of acetic acid, sour vinegar, while  
386 butyric acid odor is cheesy, rancid and that of isovaleric acid, pungent. Less odorant SCFA are  
387 on Fig 5b with lactic, oxalic, malic, tartaric, and citric acids (C2, C3, C4 and C6 respectively). In  
388 addition to carboxylic function, lactic, malic, and tartaric acids have one or two alcohol  
389 functions and oxalic and citric acids are respectively di- and tri- acids which may contribute to  
390 decreasing their volatility and therefore increasing their odor threshold. An interesting point  
391 is that the odorant SCFA were produced during fermentation while the others decreased  
392 (tartrate and citrate being the most abundant in the raw seeds) or increased and then  
393 decreased (oxalate, malate, and lactate). SCFA may have different origins. Butyric acid may  
394 come from FFA like in cheese (Faccia et al., 2018; Poveda, Justa María et al., 2008). Acetic acid  
395 may come from the oxidation of ethanol during fermentation while Isovaleric acid (3-



1  
2  
3 396 methylbutanoic acid) from branched amino acids like valine, leucine, and isoleucine. Among  
4  
5 397 volatile acids, 2-methylbutanoic and 3-methylbutanoic acids were found to contribute largely  
6  
7 398 to the typical aroma of cheese or fermented ALBS (Akanni et al., 2018; Poveda, Justa María et  
8  
9 399 al., 2008). Also, the Verhulst function described odorant SCFA release well. The first acids  
10  
11 400 produced were acetic and formic acids at a slow rate followed by isovaleric and then butyric  
12  
13 401 acids that exhibited a more logistic shape (higher L parameter) and slightly higher rates of  
14  
15 402 formation (Figure 3). This order of release is concomitant with the successive consumption of  
16  
17 403 carbohydrates, proteins and lipids as substrates for *Bacillus*. Therefore, SCFA are prevalent  
18  
19 404 compounds released throughout ALBS fermentation but their individual prevalence could be  
20  
21 405 modulated by the macro-composition of ALBS and by fermentation time. This modulation  
22  
23 406 could markedly influence odor but also taste of FALBS.  
24

#### 25 408 **Evolution of the different VOCs families**

26  
27 409 A total of 111 volatile organic compounds (VOCs) were collected from the different samples:  
28  
29 410 raw seeds, and cotyledons during different times of fermentation which is consistent with the  
30  
31 411 number of 116-125 found in previous studies on similar products (Akanni et al., 2018; Ouoba  
32  
33 412 et al., 2005). A total of 13 pyrazines, 9 aldehydes, 11 ketones, 15 esters, 16 acids, 15 alcohols,  
34  
35 413 1 alkene, 1 amine, 2 pyridines, 8 benzenes, 2 phenols, 2 sulfur compounds, 6 amides, 1 furan  
36  
37 414 and 9 others were found. 60 of the most frequent VOCs constituting the 'volatolom' of our  
38  
39 415 samples of FALBS are presented in Table 1. These compounds were found in other protein-  
40  
41 416 rich products like beans, cheese, milk, shrimp, or cured fish such as anchovies or fermented  
42  
43 417 products such as coco beans, wine, or free amino acid-rich products such as mushrooms  
44  
45 418 (Aisala et al., 2019; Akanni et al., 2018; Amoa-Awua et al., 2014; Dehaut et al., 2014; Dursun  
46  
47 419 et al., 2017; Kouakou-Kouamé et al., 2020). During cocoa bean fermentation, there is a  
48  
49 420 succession of VOCs families as a function of fermentation time due to microbial succession of  
50  
51 421 a wide range of yeasts, lactic acid and acetic acid bacteria (Kuhnert et al., 2020; Schwan, 1998).  
52  
53 422 In the case of ALBS fermentation, flora is supposed to be more uniform and mainly composed  
54  
55 423 of *Bacillus* conducting an alkaline fermentation. Therefore, the study of the release kinetics of  
56  
57 424 VOCs in FALBS may contribute to explaining the metabolism behavior of *Bacillus* and its  
58  
59 425 relationship with the final aroma.  
60  
61 426 Figures 6 and 7 present the evolution of the different families of VOCs during production of  
62  
63 427 fermented ALBS. The main constituents of raw seeds were alcohols (~30%), acids and esters.

1  
2  
3 428 After cooking, a marked change occurred with an increase of pyrazines from 10 to nearly 60  
4  
5 429 % of VOCs. This increase was certainly due to the Maillard reactions during cooking but also  
6  
7 430 the leaking of some hydrophilic or thermosensitive compounds like alcohols and esters while  
8  
9 431 the acids remained stable. After fermentation, a second marked change was observed with  
10  
11 432 the prevalence of acids that account for more than 45% of the VOCs. Pyrazines still remained  
12  
13 433 at a high concentration as can be observed in Figure 5a). Their production was not  
14  
15 434 monotonous. Indeed, their content increased at the beginning of fermentation but decreased  
16  
17 435 after 12 h so their final quantity was not significantly different from that at the beginning. The  
18  
19 436 total of VOCs increased significantly during fermentation ( $p < 0.05$ ) and were strongly positively  
20  
21 437 correlated to the production of acids ( $p < 0.05$ ). As can be seen in Fig 5b), another category of  
22  
23 438 product that rose markedly during fermentation were amides ( $p < 0.05$ ). The other compounds:  
24  
25 439 alcohols, aldehydes, esters, ketones, and furans, decreased at the beginning of fermentation  
26  
27 440 (specially for aldehydes, esters and ketones) but were produced after 24 h. However, their  
28  
29 441 final content was still inferior or not significantly different to  $t=0$  h. Sulfur compounds were in  
30  
31 442 small quantity and did not evolve significantly.

32 443

#### 33 444 **Succession of VOCs during fermentation**

34 445 To be more specific about the different molecules that evolved significantly during FALBS  
35  
36 446 production, ANOVA analysis was carried out for all VOCs followed by PCA only on the  
37  
38 447 molecules that discriminated the fermentation time. The results of PCA are presented in Fig.  
39  
40 448 8. The projection of different samples showed that PCA enabled the discrimination of raw  
41  
42 449 seeds (RS) from the cooked cotyledons before fermentation (0 H). The typical VOCs that were  
43  
44 450 positively correlated with the raw seeds were: ethanol, hexanol, carbon disulfide, butanal and  
45  
46 451 4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one. This last molecule is also called verbenone and  
47  
48 452 has a camphoreous odor (Table 1). Alcohols are typical of the raw seeds and among them 1-  
49  
50 453 hexanol which is responsible for green notes than can be found in peas for instance (C. Zhang  
51  
52 454 et al., 2020).

53 455 These compounds were lost significantly during cooking and may therefore contribute only  
54  
55 456 slightly to the flavor of the final product. However, some VOCs were positively correlated with  
56  
57 457  $t=0$  hours and therefore may have appeared during cooking. Among them, one pyrazine, 2,3-  
58  
59 458 Dimethyl-5-ethylpyrazine that has a strong roasted aroma, could have come from the long  
60  
459 cooking period (Qian & Reineccius, 2002). We also found xylene which is an aromatic

1  
2  
3 460 hydrocarbon and may be considered as contamination arising from wood-cooking (Ciecierska,  
4 461 2020). Lastly, 4-methanol-himidazole is a late Maillard compound (Licht et al., 1992). These  
5 462 two last compounds are cyclic neo-formed compounds produced because of the very long  
6 463 cooking time that may have an adverse effect on health at high concentrations.

7 464 The compounds positively correlated with the first hours of fermentation were aldehyde  
8 465 (benzaldehyde and 2,5-dihydroxybenzaldehyde), esters (ethyl acetate), ketones (acetoin, 2,3  
9 466 butadione, 2-decanone and 2-octanone), 2-pentyl-thiophene and 2-pentyl-Furan. 2-  
10 467 pentylfuran and 1-octen-3-ol compounds are known for contributing to the green and beany  
11 468 aroma of cooked beans (Owens et al., 1997). They were also found in sugar-rich fermented  
12 469 products such as grape wine, alcoholic beverages made from sorghum and particularly fruit  
13 470 wines involving yeasts (Feng et al., 2015; Song et al., 2019; H. Zhang et al., 2021). Therefore,  
14 471 these compounds may come from the cooking process and the early stage of fermentation  
15 472 when *Bacillus* consume sugars and provide fruity notes. Owens et al. (1997) reported that  
16 473 *Bacillus subtilis* when grown in sugars excrete alpha-acetolactate that is later decarboxylated  
17 474 to acetoin which is consumed when carbohydrate depletion occurs. He also noticed that the  
18 475 other ketones produced may be the result of lipid and/or amino acid degradation. However,  
19 476 these compounds have a relative high odor threshold so they may only contribute slightly to  
20 477 the aroma of FALBS.

21 478 The other group that appeared during fermentation were pyrazines. Their maximum content  
22 479 was achieved between 10 to 15 h of fermentation. The main pyrazines by order of quantity  
23 480 were 2,5-dimethyl-pyrazine, methyl-pyrazines, ethyl-pyrazine, 2-ethyl-6-methyl-pyrazine, and  
24 481 2,6-dimethyl-pyrazine. Pyrazines in general and 2,5-dimethyl-pyrazine in particular were also  
25 482 found during *Bacillus* fermentations (Akanni et al., 2018; Besson et al., 1997; Ouoba et al.,  
26 483 2005; Owens et al., 1997). Owen et al. (1997) also noticed that the free amino acids, the  
27 484 alkaline pH and the incubation temperature (40 °C) favored the production of pyrazines.  
28 485 Ouoba et al. (2003), during fermentation in controlled conditions in the laboratory with  
29 486 different *Bacillus* starters, found that the profile of pyrazines, ketones, aldehydes and esters  
30 487 was dependent on the *Bacillus* species. In our case, we observed that the amount of pyrazine  
31 488 significantly decreased at the end of fermentation until it reached the initial level just after  
32 489 cooking. This decrease was also noticed but not explained by Owen et al. (1997). Therefore,  
33 490 the roasted notes of the final product due to pyrazines may depend on the fermentation time.

1  
2  
3 491 Contrary to pyrazines, acids were released constantly throughout fermentation. The main acid  
4  
5 492 in quantity was 3-methyl-butanoic acid (isovalerate) as observed previously, followed by 2-  
6  
7 493 methyl-butanoic acid. In addition, with these two volatile acids, we found 2-methyl-2-butenic  
8  
9 494 acid also called tiglic acid. It is supposed to be formed from isoleucine and presents ripe fruit  
10  
11 495 notes (Y. Zhang et al., 2018). Simultaneously with acids, esters were formed. Akanni et al.  
12  
13 496 (2018) noticed that esters have been known to constitute a major volatile compound in  
14  
15 497 African fermented condiments. The esters are presumably the consequence of chemical  
16  
17 498 reactions between acids and alcohols and produce floral and fruity notes. Logically, the main  
18  
19 499 esters found were that of the main acids, i.e., 3-methyl-butanoic ester, 2-methyl-butanoic  
20  
21 500 ester, and acetic acid-methyl ester. The other secondary products released were amides that  
22  
23 501 were correlated to the last hours of fermentation. The main amides were acetamide,  
24  
25 502 propanamide, butanamide, 2-methyl-propanamide and 3-methyl-butanamide. These  
26  
27 503 molecules may come from the reaction between free amino acids and acids and were  
28  
29 504 observed in cocoa beans and wine (Kuhnert et al., 2020; Schueuermann et al., 2016).  
30  
31 505 Therefore, acids are involved in different reactions with alcohols and free amino acids which  
32  
33 506 may explain the slight decrease of certain acids like formate or malate at the end of  
34  
35 507 fermentation as observed in Fig 5a and b. Lastly, two other VOCs were found at the very final  
36  
37 508 stage, which were phenol, a degradation product of tyrosine, and 2-piperidinone found in  
38  
39 509 fermented bean (Kim & Chung, 2008; Mathus et al., 1995). Phenol is an irritant and considered  
40  
41 510 objectionable while 2-piperidinone, is ammoniacal and pepper-like (Maraval et al., 2008).  
42  
43 511 Again, specific monitoring of the last hours of fermentation could be of interest to control the  
44  
45 512 concentration of these two last compounds.

513

## 514 Conclusion

515 This paper showed the release of different bioactive compounds during spontaneous FALBS  
516 production that have sensory, nutritional and toxicity impacts. During fermentation, VOCs  
517 number was multiplied by a ten-fold which confirm the very important impact of this step on  
518 the final quality of FALBS. This is because ALBS is a legume rich in proteins and lipids and  
519 because *Bacillus* species involved in this fermentation are able to metabolize them with their  
520 proteases and lipases. The results also highlighted the important kinetic aspects of this  
521 fermentation. The main early products are a result of protein catabolism and are free amino  
522 acids with glutamic acid presenting the highest release and some biogenic amines like

1  
2  
3 523 cadaverine, putrescine and histamine. In a second phase, SCFA and FFA releases occurred.  
4  
5 524 Particularly, the cleavage of branched-chain amino acids produced isovaleric acid which was  
6  
7 525 the main acid produced. The odorant butyric acid was produced later and at a lower content  
8  
9 526 certainly due to triglyceride hydrolysis. Therefore, we can conclude that the first 20 hours of  
10  
11 527 fermentation of ALBS lead to maximal contents in acids contributing significantly to the “sour,  
12  
13 528 pungent and cheesy” notes of FALBS. During this phase, pyrazines were also produced in high  
14  
15 529 certainly giving roasted notes to FALBS. Lastly, esters, amides and phenol were produced from  
16  
17 530 complex reactions between amino acids, alcohols and acids produced in the previous phases.  
18  
19 531 Maximal ammonia content was achieved during this phase increasing the final pH of FALBS.  
20

21  
22 532  
23 533 The perspectives of this research will be to work firstly on the raw material. Indeed, raw seed  
24  
25 534 composition and their pre-treatment (cooking) are important to monitor the proportion of  
26  
27 535 carbohydrates, proteins and lipids that are the substrates of fermentation. Secondly, it would  
28  
29 536 be important to deepen the link between *Bacillus* species growth and the bioactive  
30  
31 537 compounds release during fermentation. To do so, a controlled fermentation should be done  
32  
33 538 and *Bacillus* population, enzyme activities, AA, BA, SCFA, and VOCs should be monitored as a  
34  
35 539 function of fermentation time. Lastly, the link between fermentation conditions (temperature,  
36  
37 540 aeration) and these dynamics would be very crucial to make in order to understand the  
38  
39 541 possible levers of bioactive compounds modulation in the final product.  
40

41

#### 42 542 **Conflicts of interest statement**

43 543  
44 544 The authors declare that they have no known competing financial interests or personal  
45  
46 545 relationships that could have appeared to influence the work reported in this paper.  
47

48

#### 49 546 **Figure and Table list**

50 547 **Figure 1. Evolution of macro-constituents during fermentation of ALBS**

51 548 **Figure 2. Release in g/100 g DB of a) free amino acids (AA), b) ammonium (NH<sub>4</sub><sup>+</sup>), c) free fatty**  
52  
53 549 **acids (FFA), d) biogenic amines (BA), e) short chain fatty acids (SCFA) during ALBS**  
54  
55 550 **fermentation. Error bars represent the standard deviation (n = 3) and lines represent the**  
56  
57 551 **modelled data.**

58 552 **Figure 3. Verhulst equation parameters for all compound releases during fermentation of**  
59  
60 553 **ALBS**

1  
2  
3 555 **Figure 4. Release of a), b), c) free amino acids and d) biogenic amines during ALBS**  
4  
5 556 **fermentation. Error bars represent the standard deviation (n = 3) and lines represent the**  
6  
7 557 **modelled data.**

8 558 **Figure 5. Release of a) odorant SCFA and d) less odorant SCFA during ALBS fermentation.**  
9  
10 559 **Error bars represent the standard deviation (n = 3) and plain lines represent the modelled**  
11  
12 560 **data.**

13  
14 561 **Figure 6. Evolution of the different VOCs according to the chemical family during fermented**  
15  
16 562 **ALBS production**

17  
18 563 **Figure 7. Evolution of a) total VOCs, acids and pyrazines and b) other VOCs families during**  
19  
20 564 **ALBS fermentation**

21 565 **Figure 8. PCA on molecules that evolve significantly during FALBS production (RS: raw seed)**

22  
23 566 **Table 1. Most frequent VOCs produced during fermented ALB production**  
24

25 567  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

568

569 **References**

570

571 Adeyemo, S. M., &amp; Onilude, A. A. (2013). Enzymatic Reduction of Anti-nutritional Factors in

572 Fermenting Soybeans by *Lactobacillus plantarum* Isolates from Fermenting Cereals. *Nigerian*573 *Food Journal*, 31(2), 84–90. [https://doi.org/10.1016/S0189-7241\(15\)30080-1](https://doi.org/10.1016/S0189-7241(15)30080-1)

574 Aisala, H., Sola, J., Hopia, A., Linderborg, K. M., &amp; Sandell, M. (2019). Odor-contributing volatile

575 compounds of wild edible Nordic mushrooms analyzed with HS–SPME–GC–MS and HS–

576 SPME–GC–O/FID. *Food Chemistry*, 283, 566–578.577 <https://doi.org/10.1016/j.foodchem.2019.01.053>

578 Akanni, G. B., De Kock, H. L., Naudé, Y., &amp; Buys, E. M. (2018). Volatile compounds produced by

579 *Bacillus* species alkaline fermentation of bambara groundnut (*Vigna subterranean* (L.) Verdc)

580 into a dawadawa-type African food condiment using headspace solid-phase microextraction

581 and GC × GC–TOFMS. *International Journal of Food Properties*, 21(1), 930–942.582 <https://doi.org/10.1080/10942912.2018.1460757>

583 Alfaia, C. M., Castro, M. F., Reis, V. A., Prates, J. M., de Almeida, I. T., Correia, A. D., &amp; Dias, M. A.

584 (2004). Changes in the Profile of Free Amino Acids and Biogenic Amines During the

585 Extended Short Ripening of Portuguese Dry-Cured Ham. *Food Science and Technology*586 *International*, 10(5), 297–304. <https://doi.org/10.1177/1082013204047597>

587 Alvarez, M. A., &amp; Moreno-Arribas, M. V. (2014). The problem of biogenic amines in fermented foods

588 and the use of potential biogenic amine-degrading microorganisms as a solution. *Trends in*589 *Food Science & Technology*, 39(2), 146–155. <https://doi.org/10.1016/j.tifs.2014.07.007>

590 Amoa-Awua, W. K., Awusi, B., Owusu, M., Appiah, V., Ofori, H., Thorsen, L., &amp; Jespersen, L. (2014).

591 Reducing the atypical odour of dawadawa: Effect of modification of fermentation conditions

592 and post-fermentation treatment on the development of the atypical odour of dawadawa.

593 *Food Control*, 42, 335–342. <https://doi.org/10.1016/j.foodcont.2014.02.016>

- 1  
2  
3 594 Azokpota, P., Hounhouigan, D. J., & Nago, M. C. (2006). Microbiological and chemical changes during  
4  
5 595 the fermentation of African locust bean (*Parkia biglobosa*) to produce afitin, iru and sonru,  
6  
7 596 three traditional condiments produced in Benin. *International Journal of Food Microbiology*,  
8  
9 597 *107*(3), 304–309. <https://doi.org/10.1016/j.ijfoodmicro.2005.10.026>  
10  
11  
12 598 Besson, I., Creuly, C., Gros, J. B., & Larroche, C. (1997). Pyrazine production by *Bacillus subtilis* in  
13  
14 599 solid-state fermentation on soybeans. *Applied Microbiology and Biotechnology*, *47*(5), 489–  
15  
16 600 495. <https://doi.org/10.1007/s002530050961>  
17  
18  
19 601 Çalık, P., Çalık, G., & Özdamar, T. H. (2000). Oxygen-transfer strategy and its regulation effects in  
20  
21 602 serine alkaline protease production by *Bacillus licheniformis*. *Biotechnology and*  
22  
23 603 *Bioengineering*, *69*(3), 301–311. <https://doi.org/10.1002/1097->  
24  
25 604 [0290\(20000805\)69:3<301::AID-BIT8>3.0.CO;2-4](https://doi.org/10.1002/1097-0290(20000805)69:3<301::AID-BIT8>3.0.CO;2-4)  
26  
27  
28 605 Ciecierska, M. (2020). Cocoa beans of different origins and varieties and their derived products  
29  
30 606 contamination with polycyclic aromatic hydrocarbons. *Food Chemistry*, *317*, 126408.  
31  
32 607 <https://doi.org/10.1016/j.foodchem.2020.126408>  
33  
34  
35 608 Cohen, G., Laloush, M., & Karpas, Z. (2014). Biogenic amines in bread as indicators of spoilage.  
36  
37 609 *International Journal for Ion Mobility Spectrometry*, *17*(3), 125–129.  
38  
39 610 <https://doi.org/10.1007/s12127-014-0159-3>  
40  
41  
42 611 Corrales, C. V., Lebrun, M., Vaillant, F., Madec, M. N., Lortal, S., Pérez, A. M., & Fliedel, G. (2017). Key  
43  
44 612 odor and physicochemical characteristics of raw and roasted jicaro seeds (*Crescentia alata*  
45  
46 613 K.H.B.). *Food Research International*, *96*, 113–120.  
47  
48 614 <https://doi.org/10.1016/j.foodres.2017.03.009>  
49  
50  
51 615 Dakwa, S., Sakyi-Dawson, E., Diako, C., Annan, N. T., & Amoa-Awua, W. K. (2005). Effect of boiling and  
52  
53 616 roasting on the fermentation of soybeans into dawadawa (soy-dawadawa). *International*  
54  
55 617 *Journal of Food Microbiology*, *104*(1), 69–82.  
56  
57 618 <https://doi.org/10.1016/j.ijfoodmicro.2005.02.006>  
58  
59  
60



- 1  
2  
3 619 Dehaut, A., Himber, C., Mulak, V., Grard, T., Krzewinski, F., Le Fur, B., & Duflos, G. (2014). Evolution of  
4  
5 620 Volatile Compounds and Biogenic Amines throughout the Shelf Life of Marinated and Salted  
6  
7 621 Anchovies (*Engraulis encrasicolus*). *Journal of Agricultural and Food Chemistry*, 62(32), 8014–  
8  
9 622 8022. <https://doi.org/10.1021/jf5021736>
- 11  
12 623 Dekeirsschietter, J., Verheggen, F. J., Gohy, M., Hubrecht, F., Bourguignon, L., Lognay, G., & Haubruge,  
13  
14 624 E. (2009). Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus*  
15  
16 625 *domesticus* L.) in different biotopes. *Forensic Science International*, 189(1), 46–53.  
17  
18 626 <https://doi.org/10.1016/j.forsciint.2009.03.034>
- 20  
21 627 Delgado, F. J., González-Crespo, J., Cava, R., & Ramírez, R. (2011). Free Fatty Acids and Oxidative  
22  
23 628 Changes of a Raw Goat Milk Cheese through Maturation. *Journal of Food Science*, 76(4),  
24  
25 629 C669–C673. <https://doi.org/10.1111/j.1750-3841.2011.02140.x>
- 27  
28 630 Dursun, A., Güler, Z., & Şekerli, Y. E. (2017). Characterization of volatile compounds and organic acids  
29  
30 631 in ultra-high-temperature milk packaged in tetra brik cartons. *International Journal of Food*  
31  
32 632 *Properties*, 20(7), 1511–1521. <https://doi.org/10.1080/10942912.2016.1213280>
- 34  
35 633 Esenwah, C., & Ikenebomeh, M. (2008). Processing effects on the nutritional and anti-nutritional  
36  
37 634 contents of African locust bean (*Parkia biglobosa* Benth.) seed. *Pakistan Journal of Nutrition*,  
38  
39 635 7(2), 214–217.
- 41  
42 636 Esse, M. Y., Guehi, T. S., Grabulos, J., Morel, G., Malomar, R. T., Tardan, E., Mestres, C., & Achir, N.  
43  
44 637 (2021). Fate of proteic and lipidic compounds during production of a traditional legume  
45  
46 638 condiment (Soumbala) made from African Locust Bean (*Parkia biglobosa*) seeds.  
47  
48 639 *International Journal of Food Science & Technology*, 56(2), 804–813.  
49  
50 640 <https://doi.org/10.1111/ijfs.14724>
- 52  
53 641 Faccia, M., Trani, A., Natrella, G., & Gambacorta, G. (2018). Short communication: Chemical-sensory  
54  
55 642 and volatile compound characterization of ricotta forte, a traditional fermented whey  
56  
57 643 cheese. *Journal of Dairy Science*, 101(7), 5751–5757. <https://doi.org/10.3168/jds.2018-14424>  
58  
59  
60

- 1  
2  
3 644 FAO/WHO/UNU. (2007). Protein and amino acid requirements in human nutrition : report of a joint  
4  
5 645 FAO/WHO/UNU expert consultation. *WHO Technical Report Series*, 935, 265.  
6  
7 646 Feng, Y., Liu, M., Ouyang, Y., Zhao, X., Ju, Y., & Fang, Y. (2015). Comparative study of aromatic  
8  
9 647 compounds in fruit wines from raspberry, strawberry, and mulberry in central Shaanxi area.  
10  
11 648 *Food & Nutrition Research*, 59(1), 29290. <https://doi.org/10.3402/fnr.v59.29290>  
12  
13  
14 649 Granito, M., Frias, J., Doblado, R., Guerra, M., Champ, M., & Vidal-Valverde, C. (2002). Nutritional  
15  
16 650 improvement of beans (*Phaseolus vulgaris*) by natural fermentation. *European Food*  
17  
18 651 *Research and Technology*, 214(3), 226–231. <https://doi.org/10.1007/s00217-001-0450-5>  
19  
20  
21 652 Huet, S., Jolivet, E., & Messéan, A. (1992). *La régression non-linéaire: méthodes et applications en*  
22  
23 653 *biologie*.  
24  
25 654 Ito, H., Ueno, H., & Kikuzaki, H. (2018). Free Amino Acid Compositions for Fruits. *Journal of Nutrition*  
26  
27 655 *and Dietetic Practice*. <http://sciaeon.org/JNDP/free-amino-acid-compositions-for-fruits.php>  
28  
29  
30 656 Kim, J. S., & Chung, H. Y. (2008). Components in Commercial Douchi—a Chinese Fermented Black  
31  
32 657 Bean Product by Supercritical Fluid Extraction. *Journal of Food Science and Nutrition*, 12–17.  
33  
34 658 Kouakou-Kouamé, C. A., N’guessan, F. K., Montet, D., & Djè, M. K. (2020). Biogenic Amine, Fatty Acid,  
35  
36 659 and Volatile Compound Contents in Ivorian Traditionally Fermented Fish “Adjuevan.”  
37  
38 660 *Preventive Nutrition and Food Science*, 25(1), 98–107. PubMed.  
39  
40 661 <https://doi.org/10.3746/pnf.2020.25.1.98>  
41  
42  
43 662 Krishnan, H. B. (2005). Engineering Soybean for Enhanced Sulfur Amino Acid Content. *Crop Science*,  
44  
45 663 45(2), 454–461. <https://doi.org/10.2135/cropsci2005.0454>  
46  
47  
48 664 Kuhnert, N., D’souza, R. N., Behrends, B., Ullrich, M. S., & Witt, M. (2020). Investigating time  
49  
50 665 dependent cocoa bean fermentation by ESI-FT-ICR mass spectrometry. *Food Research*  
51  
52 666 *International*, 133, 109209. <https://doi.org/10.1016/j.foodres.2020.109209>  
53  
54  
55 667 Lee, Y.-C., Kung, H.-F., Huang, C.-Y., Huang, T.-C., & Tsai, Y.-H. (2016). Reduction of histamine and  
56  
57 668 biogenic amines during salted fish fermentation by *Bacillus polymyxa* as a starter culture.  
58  
59  
60

- 1  
2  
3 669 *Journal of Food and Drug Analysis*, 24(1), 157–163.  
4  
5 670 <https://doi.org/10.1016/j.jfda.2015.02.002>  
6  
7 671 Licht, B. H., Shaw, K., Smith, C., Mendoza, M., Orr, J., & Myers, D. V. (1992). Characterization of  
8  
9 Caramel Colours I, II and III. *Food and Chemical Toxicology*, 30(5), 375–382.  
10 672  
11 [https://doi.org/10.1016/0278-6915\(92\)90063-Q](https://doi.org/10.1016/0278-6915(92)90063-Q)  
12 673  
13 674 Lioe, H. N., Selamat, J., & Yasuda, M. (2010). Soy Sauce and Its Umami Taste: A Link from the Past to  
15  
16 Current Situation. *Journal of Food Science*, 75(3), R71–R76. <https://doi.org/10.1111/j.1750->  
17 675  
18 3841.2010.01529.x  
19 676  
20 677 LU, M., AN, H., & WANG, D. (2017). Characterization of Amino Acid Composition in Fruits of Three  
22  
23 Rosa roxburghii Genotypes. *Horticultural Plant Journal*, 3(6), 232–236.  
24 678  
25 <https://doi.org/10.1016/j.hpj.2017.08.001>  
26 679  
27 680 Maraval, I., Mestres, C., Pernin, K., Ribeyre, F., Boulanger, R., Guichard, E., & Gunata, Z. (2008). Odor-  
29  
30 Active Compounds in Cooked Rice Cultivars from Camargue (France) Analyzed by GC–O and  
31 681  
32 GC–MS. *Journal of Agricultural and Food Chemistry*, 56(13), 5291–5298.  
33 682  
34 <https://doi.org/10.1021/jf7037373>  
35 683  
36 684 Mathus, T. L., Townsend, D. E., & Miller, K. W. (1995). Anaerobic biogenesis of phenol and p-cresol  
38  
39 from l-tyrosine. *Fuel*, 74(10), 1505–1508. [https://doi.org/10.1016/0016-2361\(95\)00109-I](https://doi.org/10.1016/0016-2361(95)00109-I)  
40 685  
41 686 Mohamed, N., Hashim, R., Rahman, N. A., & Zain, S. M. (2001). An insight to the cleavage of [beta]-  
42  
43 carotene to vitamin A: a molecular mechanics study. *Journal of Molecular Structure:*  
44 687  
45 *THEOCHEM*, 538(1–3), 245–252.  
46 688  
47 689 Moore, S., Spackman, D. H., & Stein, W. H. (1958). Chromatography of Amino Acids on Sulfonated  
49  
50 Polystyrene Resins. An Improved System. *Analytical Chemistry*, 30, 1185–1190.  
51 690  
52 <https://doi.org/10.1021/ac60139a005>  
53 691  
54 692 Noronha, N., Cronin, D. A., O’Riordan, E. D., & O’Sullivan, M. (2008). Flavouring of imitation cheese  
55  
56 with enzyme-modified cheeses (EMCs): Sensory impact and measurement of aroma active  
57 693  
58  
59  
60

- 1  
2  
3 694 short chain fatty acids (SCFAs). *Food Chemistry*, 106(3), 905–913.  
4  
5 695 <https://doi.org/10.1016/j.foodchem.2007.06.059>  
6  
7 696 Odunfa, S. A. (1985). Biochemical changes in fermenting African locust bean (*Parkia biglobosa*) during  
8  
9 'iru' fermentation. *International Journal of Food Science & Technology*, 20(3), 295–303.  
10 697  
11 <https://doi.org/10.1111/j.1365-2621.1985.tb00379.x>  
12 698  
13  
14 699 Odunfa, S. A. (1988). African fermented foods: from art to science. *MIRCEN Journal of Applied*  
15  
16 700 *Microbiology and Biotechnology*, 4(3), 259–273. <https://doi.org/10.1007/BF01096132>  
17  
18 701 Omafuvbe, B., Falade, O., Osuntogun, B., & Adewusi, S. (2004). Chemical and biochemical changes in  
19  
20 African locust bean (*Parkia biglobosa*) and melon (*Citrullus vulgaris*) seeds during  
21 702  
22 fermentation to condiments. *Pakistan Journal of Nutrition*, 3(3), 140–145.  
23 703  
24  
25 704 Ordóñez, J. L., Callejón, R. M., Troncoso, A. M., & García-Parrilla, M. C. (2017). Evaluation of biogenic  
26  
27 705 amines profile in opened wine bottles: Effect of storage conditions. *Journal of Food*  
28  
29 706 *Composition and Analysis*, 63, 139–147. <https://doi.org/10.1016/j.jfca.2017.07.042>  
30  
31  
32 707 Ouoba, L. I. I., Diawara, B., Annan, N. T., Poll, L., & Jakobsen, M. (2005). Volatile compounds of  
33  
34 708 Soumbala, a fermented African locust bean (*Parkia biglobosa*) food condiment. *Journal of*  
35  
36 709 *Applied Microbiology*, 99(6), 1413–1421. <https://doi.org/10.1111/j.1365-2672.2005.02722.x>  
37  
38  
39 710 Ouoba, L. I. I., Rechinger, K. B., Barkholt, V., Diawara, B., Traore, A. S., & Jakobsen, M. (2003).  
40  
41 711 Degradation of proteins during the fermentation of African locust bean (*Parkia biglobosa*) by  
42  
43 712 strains of *Bacillus subtilis* and *Bacillus pumilus* for production of Soumbala. *Journal of Applied*  
44  
45 713 *Microbiology*, 94(3), 396–402. <https://doi.org/10.1046/j.1365-2672.2003.01845.x>  
46  
47  
48 714 Owens, J. D., Allagheny, N. N., Kipping, G. J., & Ames, J. M. (1997). Formation of volatile compounds  
49  
50 715 during *Bacillus subtilis* fermentation of soya beans. *Journal of the Science of Food and*  
51  
52 716 *Agriculture*, 74, 132–140.  
53  
54 717 Pasvolsky, R., Zakin, V., Ostrova, I., & Shemesh, M. (2014). Butyric acid released during milk lipolysis  
55  
56 718 triggers biofilm formation of *Bacillus* species. *International Journal of Food Microbiology*,  
57  
58 719 *181*, 19–27. <https://doi.org/10.1016/j.ijfoodmicro.2014.04.013>  
59  
60

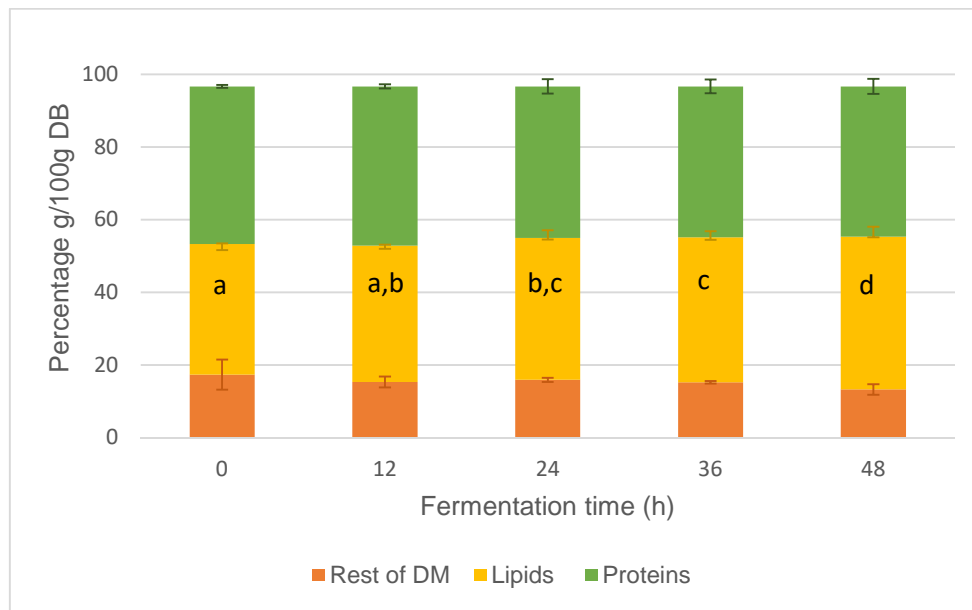
- 1  
2  
3 720 Peleg, M., Corradini, M. G., & Normand, M. D. (2007). The logistic (Verhulst) model for sigmoid  
4  
5 721 microbial growth curves revisited. *Food Research International*, *40*(7), 808–818.  
6  
7 722 <https://doi.org/10.1016/j.foodres.2007.01.012>  
8  
9  
10 723 Poveda, Justa María, Sánchez-Palomo, Eva, Pérez-Coello, María Soledad, & Cabezas, Lourdes. (2008).  
11  
12 724 Volatile composition, olfactometry profile and sensory evaluation of semi-hard Spanish goat  
13  
14 725 cheeses. *Dairy Sci. Technol.*, *88*(3), 355–367. <https://doi.org/10.1051/dst:2007021>  
15  
16 726 Qian, M., & Reineccius, G. (2002). Identification of Aroma Compounds in Parmigiano-Reggiano  
17  
18 727 Cheese by Gas Chromatography/Olfactometry. *Journal of Dairy Science*, *85*(6), 1362–1369.  
19  
20 728 [https://doi.org/10.3168/jds.S0022-0302\(02\)74202-1](https://doi.org/10.3168/jds.S0022-0302(02)74202-1)  
21  
22  
23 729 Rabie, M., Simon-Sarkadi, L., Siliha, H., El-seedy, S., & El Badawy, A.-A. (2009). Changes in free amino  
24  
25 730 acids and biogenic amines of Egyptian salted-fermented fish (Feseekh) during ripening and  
26  
27 731 storage. *Food Chemistry*, *115*(2), 635–638. <https://doi.org/10.1016/j.foodchem.2008.12.077>  
28  
29  
30 732 Reddy, N. R. (2018). *Legume Based Fermented Foods*.  
31  
32 733 Schueuermann, C., Khakimov, B., Engelsen, S. B., Bremer, P., & Silcock, P. (2016). GC-MS Metabolite  
33  
34 734 Profiling of Extreme Southern Pinot noir Wines: Effects of Vintage, Barrel Maturation, and  
35  
36 735 Fermentation Dominate over Vineyard Site and Clone Selection. *Journal of Agricultural and*  
37  
38 736 *Food Chemistry*, *64*(11), 2342–2351. <https://doi.org/10.1021/acs.jafc.5b05861>  
39  
40  
41 737 Schwan, R. F. (1998). Cocoa fermentations conducted with a defined microbial cocktail inoculum.  
42  
43 738 *Applied and Environmental Microbiology*, *64*(4), 1477–1483. PubMed.  
44  
45 739 <https://doi.org/10.1128/AEM.64.4.1477-1483.1998>  
46  
47  
48 740 Song, Z., Jia, Q., Shi, M., Feng, T., & Song, S. (2019). Studies on the Origin of Carbons in Aroma  
49  
50 741 Compounds from [13C6]Glucose -Cysteine-(E)-2-Nonenal Model Reaction Systems. *Polymers*,  
51  
52 742 *11*(3). <https://doi.org/10.3390/polym11030521>  
53  
54 743 Virgili, R., Sacconi, G., Gabba, L., Tanzi, E., & Soresi Bordini, C. (2007). Changes of free amino acids  
55  
56 744 and biogenic amines during extended ageing of Italian dry-cured ham. *LWT - Food Science*  
57  
58 745 *and Technology*, *40*(5), 871–878. <https://doi.org/10.1016/j.lwt.2006.03.024>  
59  
60

- 1  
2  
3 746 Yang, J., Rasa, E., Tantayotai, P., Scow, K. M., Yuan, H., & Hristova, K. R. (2011). Mathematical model  
4  
5 747 of *Chlorella minutissima* UTEX2341 growth and lipid production under photoheterotrophic  
6  
7 748 fermentation conditions. *Bioresource Technology*, *102*(3), 3077–3082.  
8  
9 749 <https://doi.org/10.1016/j.biortech.2010.10.049>  
10  
11  
12 750 Yoon, H., Park, J. H., Choi, A., Hwang, H.-J., & Mah, J.-H. (2015). Validation of an HPLC Analytical  
13  
14 751 Method for Determination of Biogenic Amines in Agricultural Products and Monitoring of  
15  
16 752 Biogenic Amines in Korean Fermented Agricultural Products. *Toxicological Research*, *31*(3),  
17  
18 753 299–305. PubMed. <https://doi.org/10.5487/tr.2015.31.3.299>  
19  
20  
21 754 Yusuf, O. I. S., & Rahji, M. A. Y. (2012). The Processing and Preference for Locust Bean Products  
22  
23 755 (*Parkia Biglobosa*) in Lagos, Nigeria. *Journal of Biology, Agriculture and Healthcare*, *2*.  
24  
25  
26 756 Zhan, H., Hayat, K., Cui, H., Hussain, S., Ho, C.-T., & Zhang, X. (2020). Characterization of flavor active  
27  
28 757 non-volatile compounds in chicken broth and correlated contributing constituent compounds  
29  
30 758 in muscle through sensory evaluation and partial least square regression analysis. *LWT*, *118*,  
31  
32 759 108786. <https://doi.org/10.1016/j.lwt.2019.108786>  
33  
34  
35 760 Zhang, C., Hua, Y., Li, X., Kong, X., & Chen, Y. (2020). Key volatile off-flavor compounds in peas (*Pisum*  
36  
37 761 *sativum* L.) and their relations with the endogenous precursors and enzymes using soybean  
38  
39 762 (*Glycine max*) as a reference. *Food Chemistry*, *333*, 127469.  
40  
41 763 <https://doi.org/10.1016/j.foodchem.2020.127469>  
42  
43  
44 764 Zhang, H., Wang, L., Tan, Y., Wang, H., Yang, F., Chen, L., Hao, F., Lv, X., Du, H., & Xu, Y. (2021). Effect  
45  
46 765 of *Pichia* on shaping the fermentation microbial community of sauce-flavor Baijiu.  
47  
48 766 *International Journal of Food Microbiology*, *336*, 108898.  
49  
50 767 <https://doi.org/10.1016/j.ijfoodmicro.2020.108898>  
51  
52  
53 768 Zhang, Y., Fraatz, M. A., Birk, F., Rigling, M., Hammer, A., & Zorn, H. (2018). Enantiomeric ratios of 2-  
54  
55 769 methylbutanoic acid and its methyl ester: Elucidation of novel biogenetic pathways towards  
56  
57 770 (R)-methyl 2-methylbutanoate in a beverage fermented with shiitake. *Food Chemistry*, *266*,  
58  
59 771 475–482. <https://doi.org/10.1016/j.foodchem.2018.06.027>  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

772

For Review Only



Values bearing different letters for the same parameter are significantly different ( $p < 0.05$ ).

**Figure 1. Evolution of macro-constituents during fermentation of ALBS**



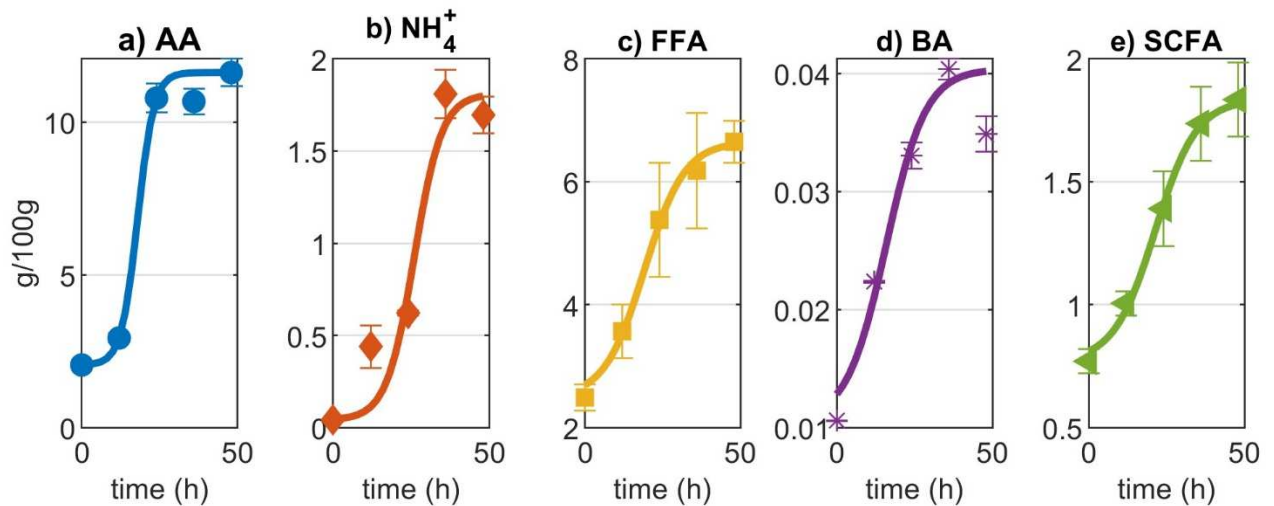
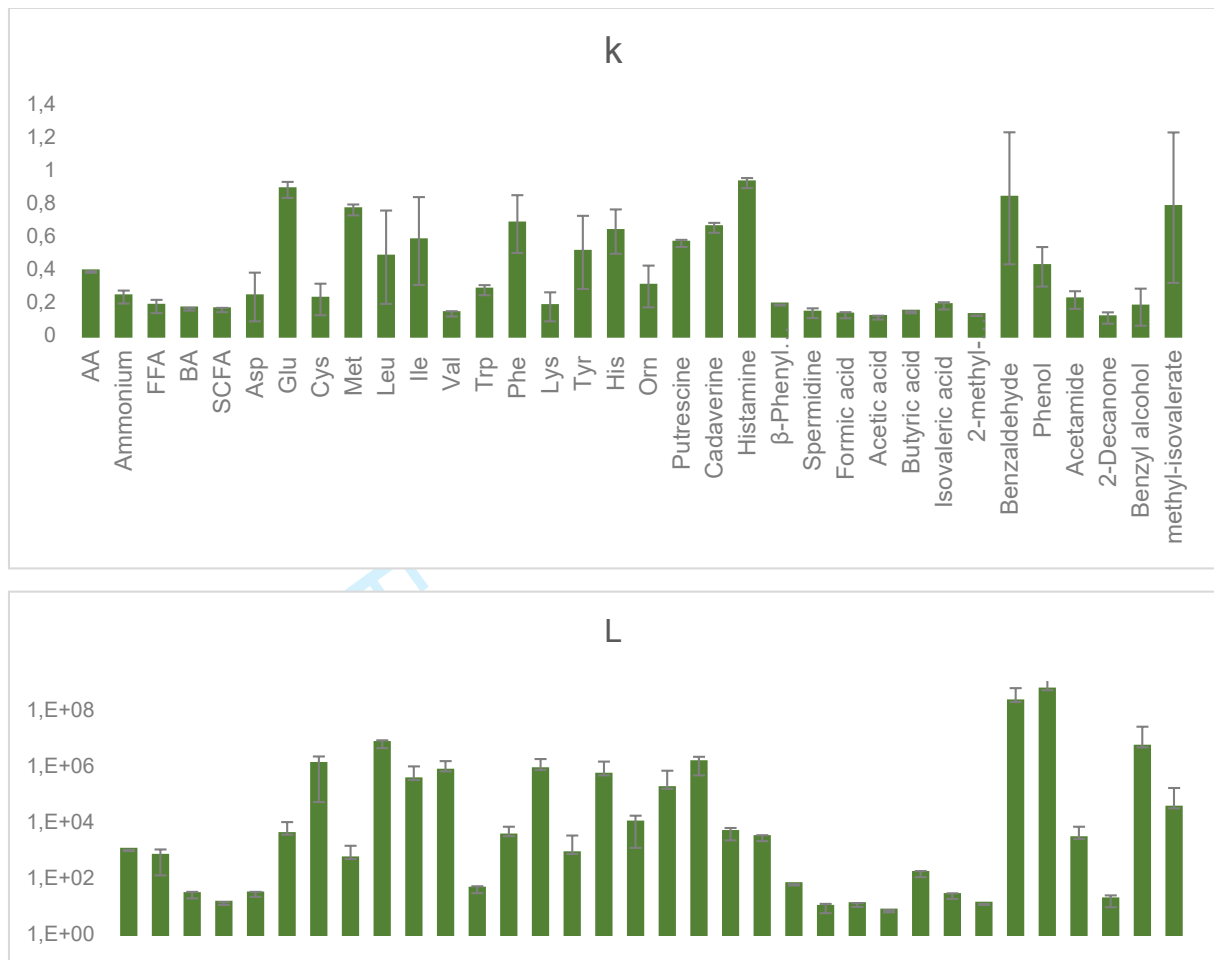
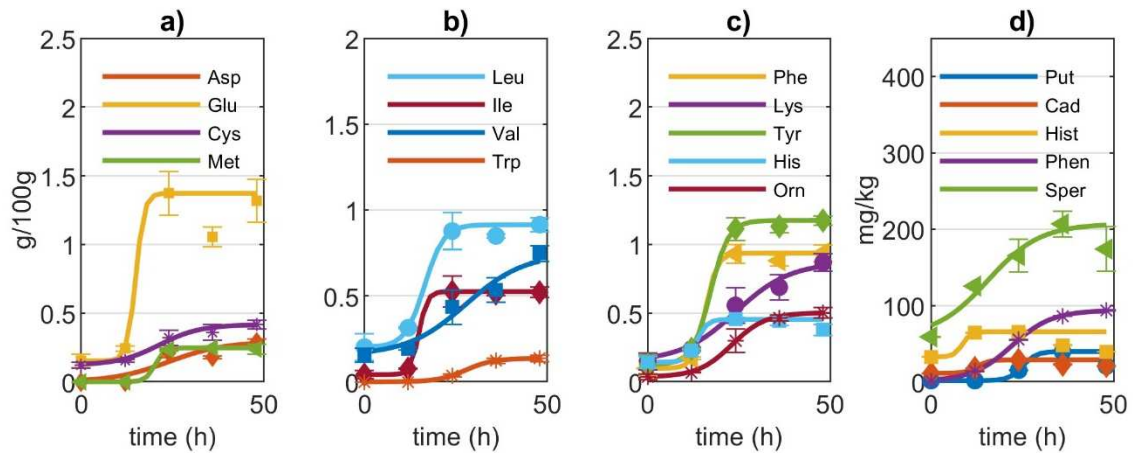


Figure 2. Release in g/100 g DB of a) free amino acids (AA), b) ammonium (NH<sub>4</sub><sup>+</sup>), c) free fatty acids (FFA), d) biogenic amines (BA), e) short chain fatty acids (SCFA) during ALBS fermentation. Error bars represent the standard deviation (n = 3) and lines represent the modelled data.

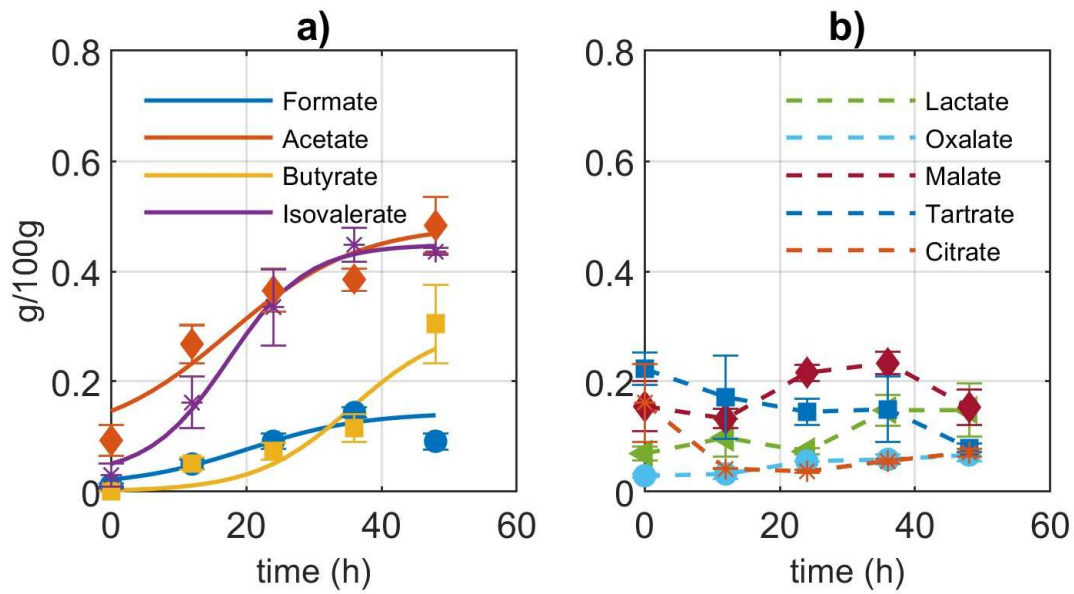


**Figure 3. Verhulst equation parameters for all compound releases during fermentation of ALBS**

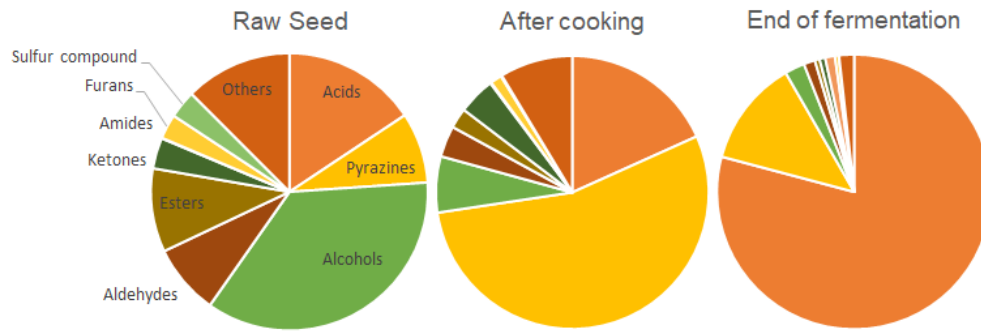


**Figure 4. Release of a), b), c) free amino acids and d) biogenic amines during ALBS fermentation. Error bars represent the standard deviation (n = 3) and lines represent the modelled data.**

For Review Only

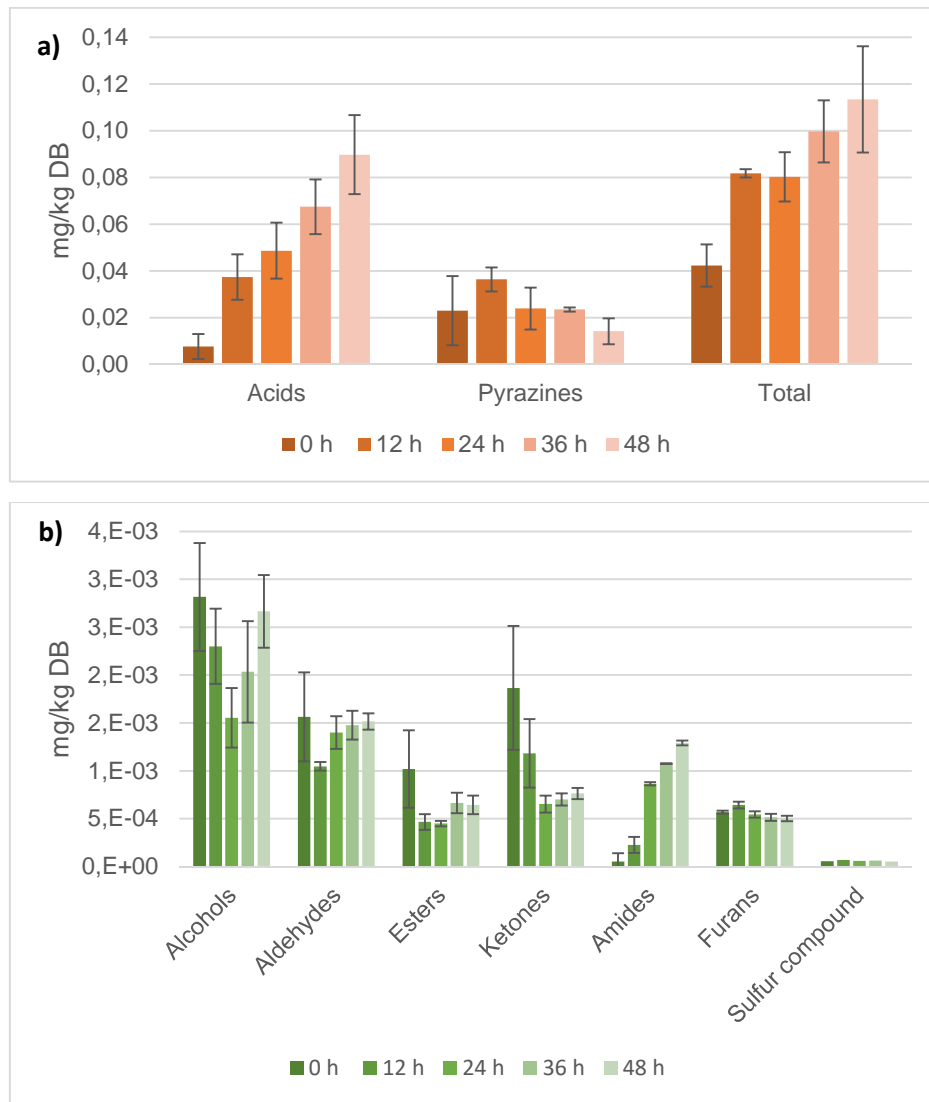


**Figure 5. Release of a) odorant SCFA and d) less odorant SCFA during ALBS fermentation. Error bars represent the standard deviation (n = 3) and plain lines represent the modelled data.**



**Figure 6. Evolution of the different VOCs according to the chemical family during fermented ALBS production**

For Review Only



**Figure 7. Evolution of a) total VOCs, acids and pyrazines and b) other VOCs families during ALBS fermentation**

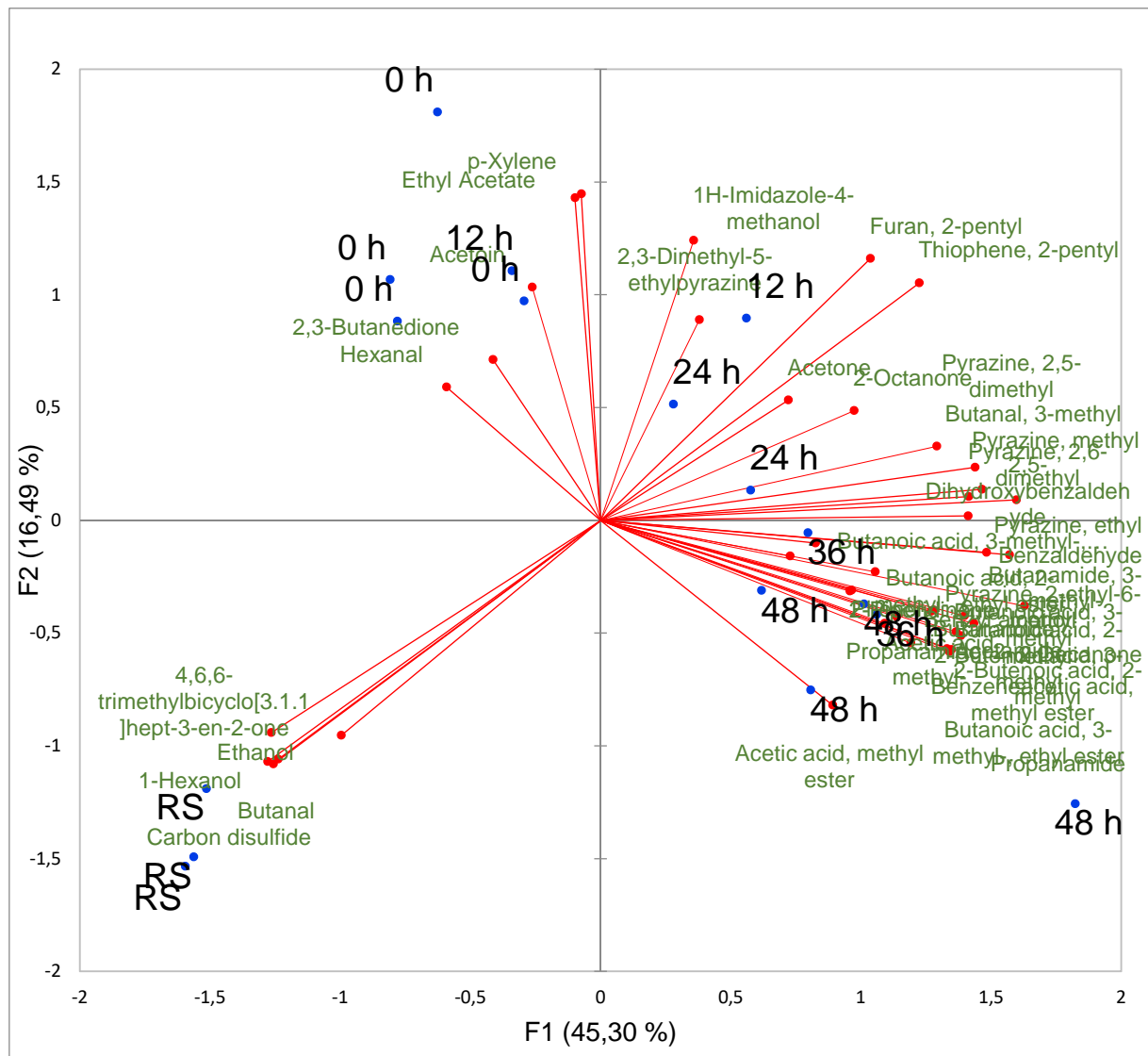


Figure 8. PCA on molecules that evolve significantly during FALBS production (RS: raw seed)

	<i>Odor</i>	<i>Assumed bioreaction origine</i>	<i>Odor threshold (µg/kg) in water</i>	<i>Product</i>	<i>References</i>	<i>Present in raw ALBS</i>	
<i>Acids</i>	3-methyl-butanoic acid (isovaleric acid)	acidic, sour, pungent,	produced from branched-chained amino acids such as valine, leucine, and isoleucine during fermentation	120	Bambara bean	Akanni (2018)	
	2-methyl-butanoic acid			100	Bambara bean	Akanni (2018)	
	2-methyl butenoic (tiglic acid)	Ripe fruit			Beverage fermented with shiitake	Zhang (2018)	
	2-methyl-propanoic acid (isobutyric acid)	Meat, cheese	-		Adjuevan	Kouakou-Kouame (2020)	
	Ethanoic acid (Acetic acid)	sour, vinegar note	Oxidation of ethanol		Bambara bean	Akanni (2018)	
	Butanoic acid (butyric acid)	rancid, cheesy			Cheese	Delgado (2011)	
	Propanoic acid (Propionic acid)	pungent acidic cheesy vinegar	hydrolysis triglycerides by lipases		Milk	Dursun (2017)	
<i>Pyrazines</i>	2,5-dimethyl-pyrazine	chocolate, roasted nut		800			
	trimethyl-pyrazine	roasted, burnt notes	from free amino acid liberation and heating	400	Bambara bean	Akanni (2018)	
	2,6-dimethyl-pyrazine	chocolate, roasted nut, fried potatoes		200			
	tetramethyl-pyrazine	nutty, musty, chocolate		1000			
<i>Alcohols</i>	1-Hexanol	Herbal, fusel oil, alcoholic	-	20000	*	yes	
	Phenylethyl Alcohol	Floral, rose, honey	-	20000	*		
	1-Octen-3-ol	mushroom and fermented-like odor	oxidation of linoleic acid		Bambara bean, Adjuevan	Akanni (2018), Kouakou-Kouame (2020)	
	1-Octanol	waxy, paint jar			mushrooms	Aisala (2019)	
	3-Octen-1-ol	fatty fruity herbal					
	Ethanol	Alcoholic	fermentation of sugars		bambara	Akanni (2018)	yes
	Benzyl alcohol	pleasant, fruity	-		bambara	Akanni (2018)	
	2-Octen-1-ol	Dust, cement, mushroom	reduction of aldehydes		Milk	Dursun (2017)	
<i>Aldehydes</i>	2,3-Butanediol	rubber, fruity, creamy, and buttery	produced from branched-chained amino acids		Bambara bean	Akanni (2018)	yes
	Benzaldehyde	pleasant, sweet, aromatic note	cysteine heating, heat generated compound, amino-acids catabolism	350	Bambara bean milk, anchovies	Akanni (2018), Dursun, Dehaut (2014)	
	Butanal (butyraldehyde)	Aldehydic, fruity	oxidative cleavage of lipids		African locust bean	Ouoba (2015)	yes
	Hexanal	green odor, fat		4.5	bambara, African locust bean, anchovies, Adjuevan	Akanni (2018), Ouoba (2015), Dehaut (2014),	yes



						Kouakou-Kouame (2020)
	3-methyl-butanal (isovaleraldehyde)	Malty, almond	oxidative cleavage of lipids, amino acid catabolism (L-Leucine)	1.1	African locust bean, anchovies	Ouoba (2015) Dehaut (2014)
<i>Esters</i>	Benzeneacetic acid methyl ester					
	Ethyl acetate					
	Acetic acid methyl ester					yes
	Methyl isovalerate - ester					
	Butanoic acid 2-methyl- ester	Floral and fruity notes	esters comes from chemical reactions between microbial acidic and alcoholic metabolites		Bambara , Adjuevan	Akanni (2018), Kouakou-Kouame (2020)
	Butanoic acid methyl ester					yes
	Acetic acid butyl ester					yes
	Butanoic acid butyl ester					yes
<i>Ketones</i>	Acetoin	butter-like aroma	Product of carbohydrates metabolism	800	bambara	Akanni (2018),
	2-Decanone	orange like floral*				
	2-Octanone	butter-like aroma*			Fruit wine	Feng (2015)    yes
	Benzyl methyl ketone					
	Acetone		products of lipids and or amino acids degradation		ALBS	Ouoba (2015)
	Acetophenone Results	sweet and floral odors, almond			Adjuevan	Kouakou-Kouame (2020)
	2,3-Butanedione	toffee, butter, candy	Fermentation of sugars		anchovies, mushrooms	Dehaut (2014), Aisala (2019)    yes
	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl	Camphoreous *				yes
<i>Amides</i>	Butanamide					
	Acetamide				Cocoa beans	Kuhnert (2020)
	N-(3-Methylbutyl) acetamide		reactions of peptides with Acids		Wine	Schueuermann (2016)
	Propanamide					
	2-methyl-propanamide,					
	3-methyl-butanamide					
<i>Others</i>	Phenol	sickening, sweet and irritating	heat-generated compounds		Milk	Dursun (2017)
	1-Butanamine					
	2-pentyl-Furan	fruity green earthy beany vegetable *			Baijiu	Zhang (2021)    yes

Carbon disulfide	pungent and sulfury odors	oxidation of the reducing sulfur compounds	bambara, Milk	Akanni (2018), Dursun (2017)	yes
1,3-dimethyl-benzene,					yes
Toluene					yes
2-pentyl-thiophene,	Wine	thermal degradation of lipid oxidation products with amino acids and reducing sugars, cysteine	Model media	Song (2019)	
Oxime methoxy-phenyl		reaction aldehydes or ketones with a nitrogen-containing reducing agent in a weakly acidic medium	Milk	Dursun (2017)	yes
Ethylbenzene	gasoline				yes
3-Acetyl-1H-pyrroline	popcorn, basmati rice		Tomato pepper pomace, mushrooms	Aisala (2019)	
Glycerin					yes
Trimethyl-oxazole	Nutty, sweet, green				
Indole	manure, burnt		Adjuevan	Kouakou-Kouame (2020)	yes
2-Piperidinone	ammoniacal, pepper-like		mushrooms	Aisala (2019)	
D-Limonene	orange*				

\*<http://www.thegoodscentcompany.com/data/rw1007681.html>

**Table 1. Most frequent VOCs produced during fermented ALB production**